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Autologization of Exosomes with Deparenchymized Adipose Tissue: An Innovative Approach for Regenerative Medicine and Surgery

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Background: Among all regenerative applications developed in recent years, the use of exosomes has generated by far the greatest interest. Exosome products from allogeneic and xenogeneic sources are available on the market. A key challenge is controlling the effects of nonautologous exosomes. We hypothesized that combining exosomes with a patient's own extracellular matrix (ECM) can create "autologization," enabling control over their effects. This study aimed to provide the rationale and a guide for future research exploring the autologization of exosome applications using deparenchymized adipose tissue (DPAT).

Methods: DPAT adipose tissue was achieved using 1200-, 400-, and 35-micrometer blades in an ultrasharp blade system (Adinizer), and then "autologization" was achieved by combining the obtained DPAT with allogeneic exosomes. DPAT was evaluated histochemically, and exosomes were counted and analyzed with the Nanosight device.

Results: The DPAT process using ultrasharp blades is easily performed. DPAT obtained from adipose tissue was then combined with allogenic exosomes. It has been demonstrated histopathologically that adipocytes are eliminated in deparenchymized fat tissue, and only ECM and stromal cells remain. It has also been proven that the number of exosomes is not affected by the combination.

Conclusions: This study introduces two novel concepts previously unknown in the literature, "deparenchymization" and "autologization," representing an innovative approach in plastic surgery and regenerative medicine. Our novel approach enriches regenerative cells while preserving critical ECM signals, overcoming the limitations of existing isolation methods. Extensive research is still needed, but autologization using DPAT ECM holds great promise for translating exosome-based treatments into practice. *(Plast Reconstr Surg Glob Open 2024; 12:e5982; doi: 10.1097/GOX.0000000000005982; Published online 16 July 2024.)*

INTRODUCTION

Regenerative medicine and surgery are rapidly advancing fields, with exosomes emerging as a promising therapeutic approach. The number of publications on exosomes has skyrocketed from 117 in 2005 to more than 32,000 by April 2024, and the volume of the exosome market will reach nearly $9 trillion by 2030.[1] However, regulatory approval and standardized protocols for exosome therapies are still lacking.

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Exosomes are complex nanovesicles that are continuously released by cells into the extracellular environment.[2] With a diameter of about 40–100 nm (Fig. 1), they contain various biomolecules that interact dynamically with the extracellular matrix (ECM).[3,4] They can be detached in all body fluids, and they express characteristics of the cell from which they were released, functioning as paracrine molecules. There is a strict relation between the ECM and exosomes.[3–5] The ECM is a complex and dynamic entity that supports and interacts with cells in tissue to regulate cell proliferation, survival, differentiation, and migration (Fig. 2).[4] Exosome products from allogeneic, animal, and plant sources are available on the market. A key challenge

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is controlling the effects of nonautologous exosomes. In their study, which presented a combination of ECM and exosomes in pulmonary fibrosis, Yu et al[4] concluded that ECM biomaterial can entrap and enrich exosomes and provide an appropriate environment for exosomes to maintain stability and function. However, obtaining the ECM in practice is a complicated process. All tissues consist of parenchymal and stromal cells. Eliminating parenchymal cells without damaging other elements leaves the stromal cells and ECM.[5] Deparenchymization of internal fat tissue can be achieved with ultrasharp blade systems.

We hypothesized that combining allogeneic or xenogeneic exosomes with an individual's own deparenchymized adipose tissue (DPAT) could enable "autologization" and provide better control over their effects (Fig. 3). This study aims to provide the rationale and proof of concept for this novel approach. As an innovative approach in plastic surgery, applying allogeneic or xenogeneic exosomes with autologous ECM may allow personalized regulation of these products. However, extensive laboratory studies, preclinical testing, and clinical trials are needed to validate this hypothesis. This study aims to provide the rationale and a guide for future research exploring the autologization of exosome applications using DPAT ECM.

Takeaways

Question: Is it possible to control the effects of ready-to-use allogeneic and xenogeneic exosomes and make them autologous?

Findings: In this study, as an innovation and new idea, the hypothesis that deparenchymized extracellular matrix (ECM) can be made from fat tissue using ultrasharp blades and that this can be used for autologization of exosomes is presented. These complementary concepts of deparenchymization and autologization represent a paradigm shift in leveraging the synergistic roles of exosomes and ECM for advanced regenerative therapies.

Meaning: Further tests and preclinical and clinical trials are needed to prove this hypothesis. However, it may be a guide for exosome treatments that are so widely and uncontrolledly used.

MATERIALS AND METHODS

To create an autologous DPAT for autologization of exosomes, we developed a method to selectively remove parenchymal adipocytes while preserving the native ECM and stromal cells. For this purpose, we used the technique defined by Copcu and Oztan,[6] in which the fat tissue is cut with ultrasharp blades, which is called adinizing (Adinizer; BSL, Inc, South Korea). However, unlike the original technique, we used only three-blade systems with different diameters: 1200, 400, and 35 µm. Under local anesthesia, 20 mL of adipose tissue was harvested and centrifuged at 500 g for 1 minute. For deparenchymization, the adipose tissue was mechanically disrupted using progressively smaller ultrasharp blades (1200, 400, and 35 µm) to selectively remove the parenchymal adipocyte cells while preserving the ECM and stromal cells. This DPAT was recentrifuged to remove triglycerides. This is the first time that the DPAT method has been described in the literature. The fat tissue was cut with relatively smaller diameter blades (35 µm) than the smallest accepted size of the adipocytes (40 µm), all parenchymal cells in the tissue were destroyed, and the final centrifugation was performed with the aim of ensuring that only ECM and stromal cells remained in the medium.

Allogeneic exosomes (Rejabeau Exo 100%; Triacle, Inc, South Korea) were combined with the DPAT to create an "autologized" exosome–ECM product. We called this technique autologization (Fig. 4: infographic). We applied the autologized exosome for aesthetic and therapeutic purposes in plastic surgery. [**See Video 1 (online)**, which displays the deparenchymization of adipose

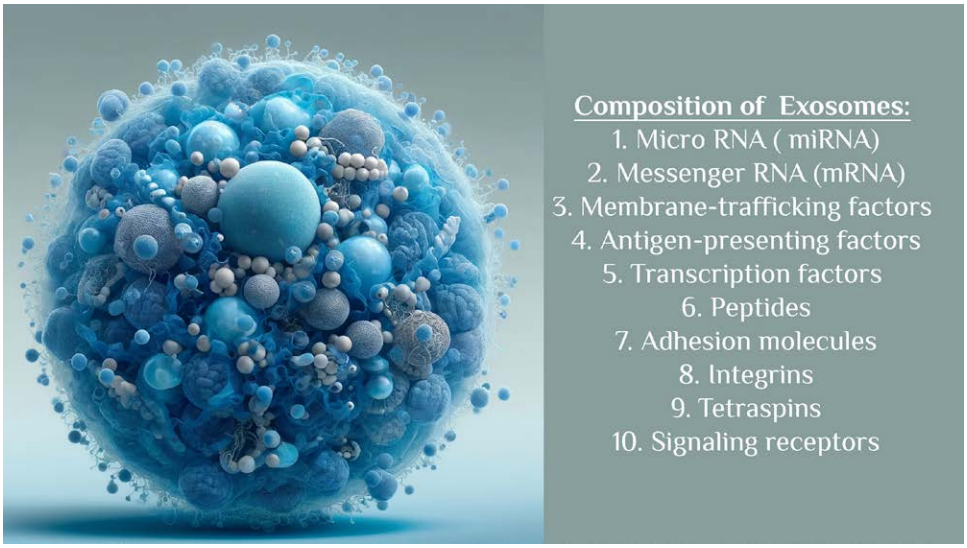

Fig. 1. Schematic view and explanations of exosomes. (This figure was created using Chat GPT 4.0 AI by entering literature information about the exosome.)

Fig. 2. Schematic view and explanations of deparenchymized ECM. (This figure was created using Chat GPT 4.0 AI by entering literature information about the ECM.)

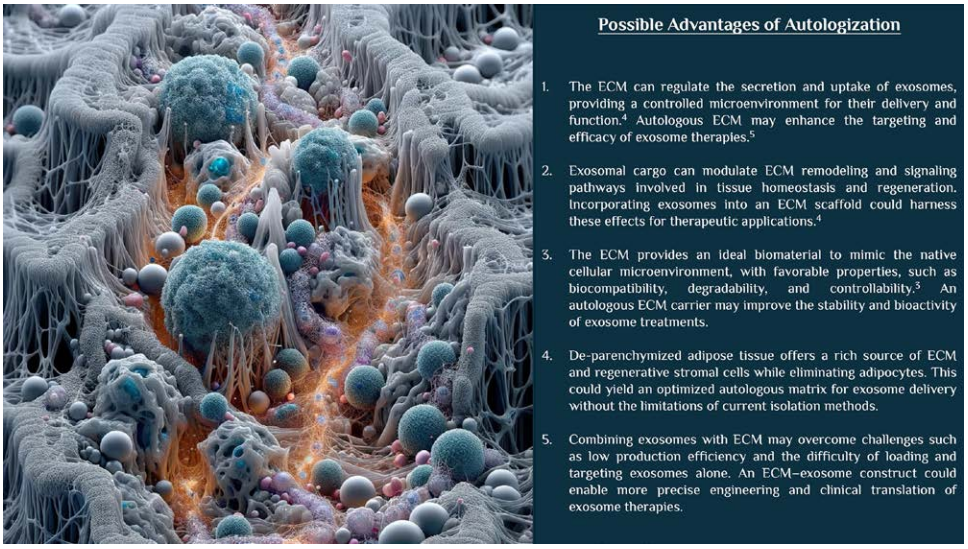

Fig. 3. Schematic view of autologization with advantages. (This figure was created using Chat GPT 4.0 AI by entering literature information about the autologization of exosome.)

tissue using ultrasharp blades (Adinizer) and exosome autologization.]

To evaluate DPAT, histopathologic examination was performed using hematoxylin–eosin (H&E) and Masson trichrome staining. Exosome counts and evaluations were conducted before and after the procedure with the Nanosight device.

RESULTS

It has been shown with two different staining techniques that even after 1200- and 400-µm adinizing procedures, adipocytes are still intact, but when a 35-µm blade is used, parenchymal cells are completely eliminated and the ECM structure is preserved. [**See figure, Supplemental Digital Content 1**, which displays the histopathologic analysis with H&E and Masson trichrome staining. A, H&E staining of adipose tissue after 1200- and 400-µm blades. Parenchymal cells (adipocytes) were still intact (200× magnification). B, Masson trichrome staining of adipose tissue after 1200- and 400-µm blades. Parenchymal cells (adipocytes) were still intact (200× magnification). C, H&E staining of adipose tissue after 1200-, 400-, and 35-µm blades. All parenchymal cells (adipocytes) were eliminated (deparenchymized), and there were only ECM (200× magnification). D, Masson trichrome staining of adipose tissue after 1200-, 400-, and 35-µm blades. All parenchymal cells (adipocytes) were eliminated (deparenchymized), and there were only ECM (200× magnification). **http://links.lww.com/PRSGO/D349**.]

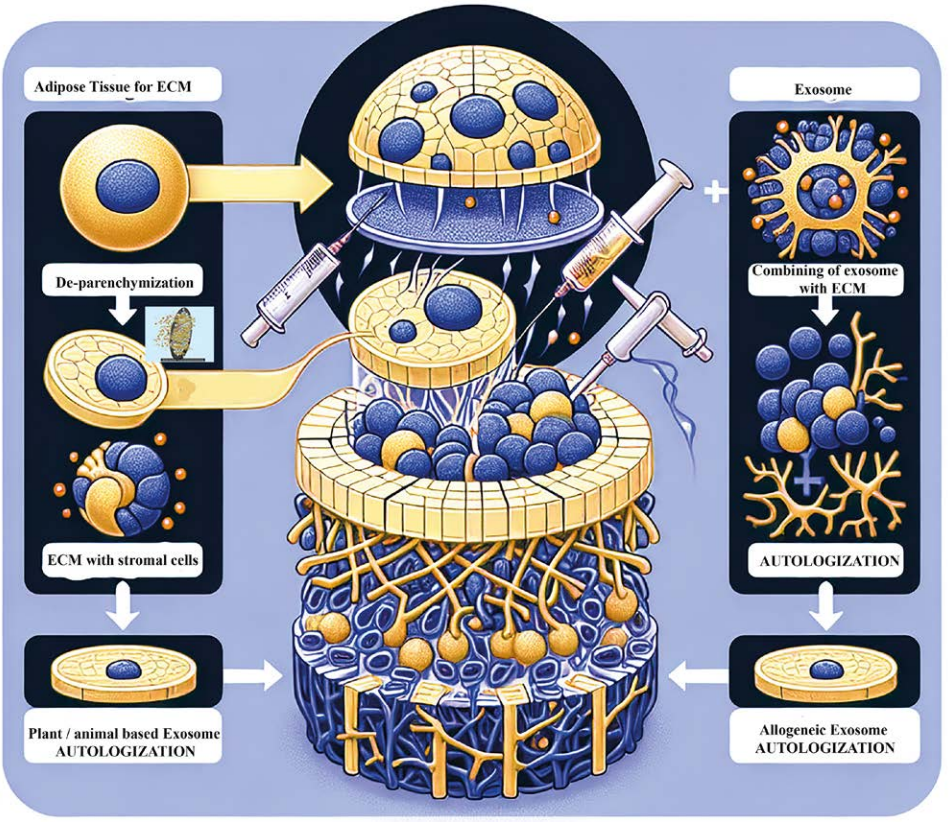

Fig. 4. Infographic of autologization for allogenic and xenogeneic exosomes. (This infographic was created using Chat GPT 4.0 AI by entering literature information about the autologization procedures.)

Exosome counts and evaluations made with the Nanosight device are presented in Figure 5. [**See Video 2 (online)**, which displays visualization of exosomes on Nanosight device.]

DISCUSSION

Regenerative applications have become very important in all medical branches. The reasons for this are the changing world order and the impact of Industry 5.0. Just as there is a transition from fossil energy to renewable energy in the energy sector, or from fast food to healthy eating choices in the food sector, there is also a transition from pharmaceutical to cell-ceutical applications in medicine. The reflections of the Industry 5.0 revolution in medicine are the "personalization and customization" approaches and the rapid introduction of regenerative practices into our routine.[7]

At the same time, providing a solution to clinical situations that are impossible or very difficult to treat has made regenerative applications much more popular. Although the beginnings of these applications date back to ancient times, among all regenerative applications, the explosive interest in exosomes has been in recent years. Although there were 117 publications on exosomes in PubMed in 2005, this number was 32,665 at the end of May 2024. This has the same effect not only in the scientific sense but also in the market sense. Globally, there are at least 204 clinical trials focusing on exosome-related studies. Of these, 114 trials are evaluating exosome-based therapeutics, and 74 trials are testing exosome-based diagnostic tests. Currently, 93 of the exosome trials (45%) are observational studies and 111 of them (55%) are interventional studies.[1]

Exosomes are nanovesicles that transport bioactive molecules to regulate cell behavior and ECM remodeling.[2–5] However, current exosome products lack ECM, which plays a crucial role in modulating their uptake, distribution, and signaling effects. The ECM is a complex network of structural and signaling molecules that control cell fate and tissue repair.[4] Incorporating autologous ECM may enhance the efficacy and safety of exosome therapies by providing a natural delivery vehicle and regulatory microenvironment.

In this study, we introduce two novel concepts for the first time in the literature, "deparenchymization" and "autologization," representing an innovative approach in plastic surgery and regenerative medicine. Deparenchymization refers to the mechanical separation and selective removal of parenchymal cells from tissue using ultrasharp blades while preserving the stromal cell population and native (ECM) architecture.[8,9] This process differs fundamentally from decellularization techniques[10] that aim to eliminate all cells to reduce antigenicity for allogeneic or xenogeneic applications. In contrast, deparenchymization enables autologization—the creation of an autologous regenerative product by removing parenchymal cells to control and modulate the

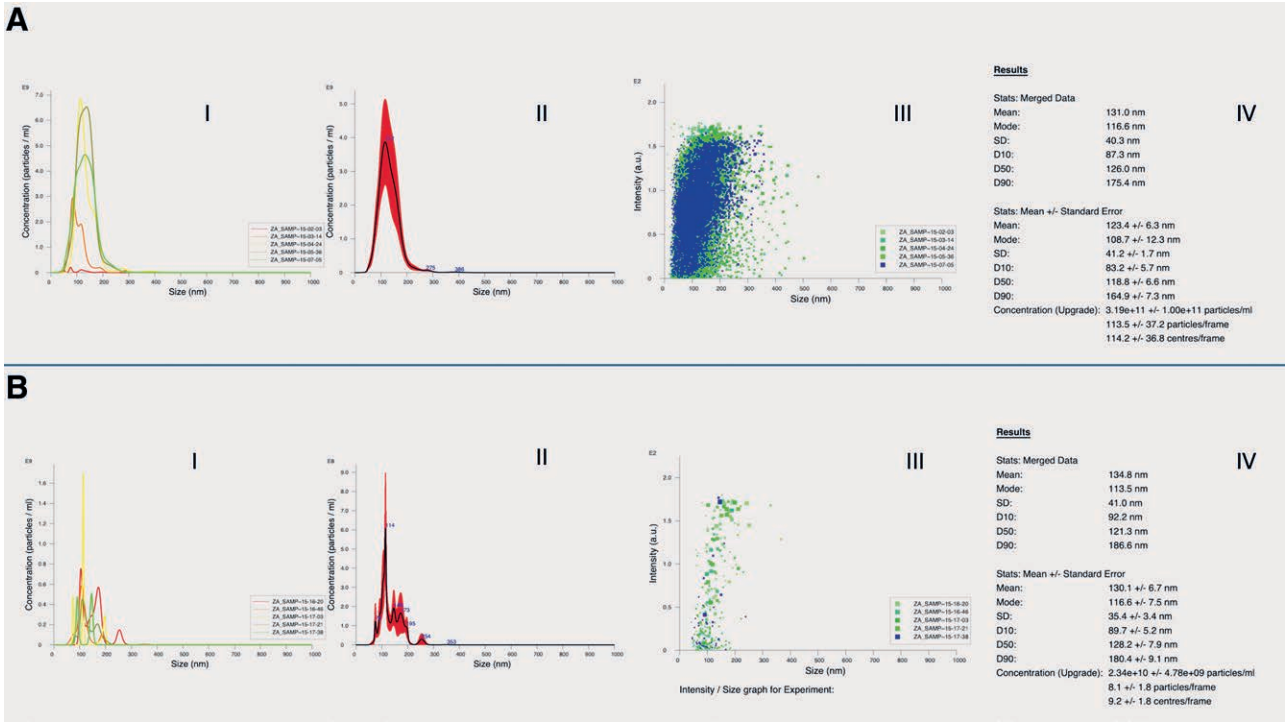

Fig. 5. Evaluation of exosomes using the Nanosight NS 300 device. Analyses were made by diluting the samples 1:500 with double distilled water. Each sample was measured five consecutive times. A, Before the process. B, After the process. I: Concentration graph according to exosome diameters of each sample (five separate measurements), II: Concentration chart evaluating exosome diameters combined, III: Density graph according to exosome sizes, and IV: Report section where all results are shown.

effects of incorporated allogeneic or xenogeneic components. By combining exogenous therapeutic agents such as exosomes with the patient's own deparenchymized tissues, autologization provides a biomimetic, personalized microenvironment to enhance delivery, bioactivity, and safety profiles compared with current cell-free formulations.

The Adinizer, a patented double-sided ultrasharp blade system, has been used in many different studies in the literature to obtain stromal cells from fat tissue and to apply different sizes to different anatomical areas in fat tissue grafts, and successful results have been reported.[11–19] The process of mechanically obtaining stromal cells using the Adinizer is called mechanical stromal cell transfer, whereas the creation of fat tissue in different sizes is called adjustable regenerative adipose transfer.[6] This approach has enabled the application of different protocols for different indications, and indication-based protocols have been presented in the literature for the first time.[20] In this way, it is possible to obtain the mechanically obtained product not only in solid form but also in liquid form for use in areas such as joints, hair, and corpus cavernosum.[20] Similarly, it has been described for the first time in the literature that mechanically obtained stromal cells in three states of matter, namely solid, liquid, and gas (aerosol), can be applied using the Adinizer,[21] and it has been shown that it can be especially effective in repairing complications of COVID-19 such as lung fibrosis.[21–23]

In the method called supercharged mechanical stromal cell transfer, which Copcu[24] described for the first time in the literature in 2021, he first mixed platelet-poor plasma, which is considered a waste and contains fibrin, with concentrated fat in the platelet-rich plasma process, and then adinized this mixture. He applied the final product, total stromal cells, by combining it with platelet-rich plasma. This approach not only enabled intensive regeneration obtained from different sources (fat tissue and blood) but also enabled regenerative cells placed in the target tissue to adhere to that area and achieve much more successful results, thanks to fibrin, a biologic glue. Finally, Copcu[25] presented a new perspective on obtaining stromal cells obtained from fat tissue in his study published in 2022. Although previously the generally accepted view was that the classification was divided into two as enzyme and mechanical methods, a clear classification has been made in this publication: parenchymal and stromal cells in adipose tissue are connected to each other very tightly with strong bridges and bonds. According to this new classification, stromal cells can be obtained by two different methods from adipose tissue. If the goal is to release these very tight bonds and bridges, this is a direct approach and can only be done with two separate methods: enzyme or ultrasharp blade systems. Either enzyme is used, these bonds are dissolved, and stromal cells are released. However, using enzymes requires special conditions from regulatory authorities (such as current good manufacturing practices, current good laboratory practice requirements,

and special permits), but more importantly, when enzymes are used, the fat tissue in the final product is considered dirty and must be disposed of. More importantly than all these restrictions, the enzyme not only dissolves bonds and bridges but also dissolves and damages ECM that must exist for regenerative applications and that we must preserve. The second direct method is the use of the Adinizer, that is, double-sided ultrasharp blades. In this method, both a higher number of stromal cells and cytokines are obtained, and the ECM is completely preserved. In addition, the fat in the final product is still intact and can be used successfully as a fat graft. Indirect methods are methods in which parenchymal tissue is eliminated and the proportion of stromal cells is relatively increased. These are microfragmentation and emulsification methods. In these methods, because the blunt pressure is high during the process, cells are damaged, and not only is the number of cells low but also their viability is very low.[25] As a result, it has been proven that the most successful approach to obtaining stromal cells from fat tissue is the use of ultrasharp blade systems. The Adinizer's successful clinical results in aesthetic and reconstructive surgery and orthopedic applications are also presented in detail in the literature.[6,11,12,19]

The use of exosomes with biologic scaffolds is very popular and has been used for many indications in the literature.[26] ECM is an ideal bioscaffold, and the use of ECM with exosomes has been successfully demonstrated in a pulmonary fibrosis model.[4]

Based on the referenced sources, the potential benefits of combining exosomes with DPAT ECM for "autologization" include the following:

1. The ECM can regulate the secretion and uptake of exosomes, providing a controlled microenvironment for their delivery and function.[4] Autologous ECM may enhance the targeting and efficacy of exosome therapies.[5]
2. Exosomal cargo can modulate ECM remodeling and signaling pathways involved in tissue homeostasis and regeneration. Incorporating exosomes into an ECM scaffold could harness these effects for therapeutic applications.[4]
3. The ECM provides an ideal biomaterial to mimic the native cellular microenvironment, with favorable properties, such as biocompatibility, degradability, and controllability.[3] An autologous ECM carrier may improve the stability and bioactivity of exosome treatments.
4. DPAT offers a rich source of ECM and regenerative stromal cells while eliminating adipocytes. This could yield an optimized autologous matrix for exosome delivery without the limitations of current isolation methods.
5. Combining exosomes with ECM may overcome challenges such as low production efficiency and the difficulty of loading and targeting exosomes alone. An ECM–exosome construct could enable more precise engineering and clinical translation of exosome therapies.

The tissue richest in ECM is adipose tissue,[8] but there has been no practical method for obtaining the ECM. In this study, adipocytes, the parenchymal cells of adipose tissue, were eliminated by the deparenchyma process using sharp blades of 1200, 400, and finally, 35 µm, and this process was called deparenchymization for the first time in the literature. This novel method selectively removes adipocytes while preserving native ECM and stromal cells.[8,9] Prior studies have shown that this yields a rich regenerative matrix with high cell viability. By combining allogeneic exosomes with autologous DPAT, we aimed to enable greater control over their therapeutic effects. Further preclinical and clinical studies are needed to validate this autologization approach. Standardized protocols and regulatory approval pathways for autologized exosome therapies must also be established. Nonetheless, leveraging the synergy between exosomes and ECM holds great promise for advancing regenerative medicine.

These complementary concepts—deparenchymization and autologization—represent a paradigm shift in leveraging the synergistic roles of exosomes and ECM for advanced regenerative therapies. Our novel approach enriches the regenerative stromal cell vascular fraction while preserving critical ECM signals, overcoming limitations of existing isolation methods. Extensive research is still needed, but autologization using DPAT ECM holds great promise for translating exosome-based treatments into clinical practice.

Exosomes are cell-free products, and these properties provide them with great advantages, especially allogeneity. However, it may create the perception of new cells joining them through the autologization phenomenon. However, this approach is very important; because in this way, the autologization process will actually turn the regenerative application into an "exosome-rich ECM" application. The person's own ECM will manage the desired regeneration, which would offer the person a great regeneration opportunity in a "healing" sense, especially if the exosome obtained from cord blood-derived mesenchymal stem cell is used. At the same time, this approach may lead to a new perspective for exosome applications in medicolegal terms.

In conclusion, autologization using DPAT shows promise for enhancing the delivery, regulation, and therapeutic potential of exosomes by providing a biomimetic and bioactive microenvironment. However, further research is needed to develop standardized protocols and to demonstrate efficacy in preclinical and clinical studies.

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DISCLOSURE

The author has no financial interest to declare in relation to the content of this article.

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